2021 BPC Symposium Abstracts

Sanjeev Kumar Memorial Lecture

Using injury kinematics of mild closed head diffuse traumatic brain injury to predict recovery outcomes in swine

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Mild traumatic brain injury (mTBI) affects millions of individuals every year primarily through falls, traffic collisions, or blunt trauma and can generate symptoms that persist for years after injury. Closed head rotational injuries are one of the most common forms of mTBI and are defined by a rapid change in acceleration within an intact skull. Injury kinematics, or the mechanical descriptors of injury motion, explain movement of the head and energy transfer to the brain, and thus, determine injury severity. However, the relationship between specific closed head rotational injury kinematics – such as velocity, acceleration, and duration – and outcome after mTBI is currently unknown. To address this gap in knowledge, we analyzed archived surgical records of swine experiencing a closed head diffuse rotational acceleration model of mTBI. Kinematics were contrasted against acute recovery outcome parameters. Thereafter, machine learning was employed to develop predictive models of recovery, informed by injury kinematics. We found that recovery was affected after a mTBI and was strongly correlated with minimum acceleration, positive acceleration duration, and total excursion. Linear discriminant analysis was able to modestly predict a delay in recovery after mTBI. Finally, we found that neuropathology was correlated with multiple kinematic parameters, but most strongly correlated to maximum acceleration. Together, these data suggest that no singular kinematic parameter was predictive of outcome, rather, interplay between multiple injury kinematics drives recovery parameters and neuropathology after mTBI in swine. Future studies designed to independently manipulate individual injury kinematics could be instrumental in developing translational diagnostics for clinical mTBI.

Platform Presentations

1. Suppression of colorectal cancer by ketogenic diet and beta-hydroxybutyrate

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Colorectal cancer (CRC) is the third deadliest cancer type, and new strategies for its prevention and therapy are urgently needed. Diet has recently emerged as an effective non-invasive intervention against neoplastic diseases but concrete recommendations for how diet can be used to prevent and treat cancer remain sparse. It is therefore critical to uncover diet-induced endogenous mechanisms that have evolved to suppress tumorigenesis. Here, we screened several different diets for their impact on CRC in mice. We found that ketogenic diet (KD) exerts a profound tumor-suppressive effect across different treatment modalities in both genetically-driven and colitis-associated CRC models. We observed that this endogenous tumor-inhibitory mechanism can be stimulated through a supplementation of the ketone body beta-hydroxybutyrate (BHB). We discovered a new function for BHB in enhancing the expression of the tumor suppressor gene Hopx, which is critical for its anti-proliferative and tumor-inhibitory effect. Also, the KD and BHB reduce the proliferation of colonic crypt within Lgr5+cells niche. Importantly, BHB potently inhibits the growth of human intestinal CRC organoids, and both KD consumption and high serum levels of BHB are associated with protection from intestinal carcinogenesis in several large-scale human cohort studies. Our findings indicate that the growth-inhibitory effect of BHB may represent one of many diet-inducible pathways by which rewiring of systemic metabolism controls peripheral tumor growth. It is possible that such dietary and metabolite-mediated circuits act synergistically with chemo- or immunotherapy and could thus offer supportive or complementary options for combination treatments.

2. Mouse model sheds light on the molecular mechanisms underlying histopathology of pulmonary Lymphangioleiomyomatosis (LAM)

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Lymphangioleiomyomatosis (LAM) is a rare fatal female-specific cystic lung disease associated with inactivating mutations in tuberous sclerosis complex (TSC1/TSC2) genes encoding the suppressors of the mechanistic target of rapamycin complex 1 (mTORC1). It is characterized by uncontrolled smooth muscle-like cell growth in the lungs, cystic destruction of the normal lung parenchyma, and lung function decline, leading to respiratory failure. The exact cell types contributing to the LAM lesions, and the mechanisms of their formation are still unclear. LAM lung single-cell profiling compared to an age- and sex-matched healthy control identified a mesenchymal cell hub that may coordinate the LAM disease phenotype. Mesenchymal-restricted deletion of Tsc2 in the mouse lung produced a mTORC1-driven pulmonary phenotype, with a progressive decline in pulmonary function and pathological structural changes in lung parenchyma that were exacerbated by pregnancies. We found increased expression of WNT ligands, as well as profound female-specific changes in mesenchymal and epithelial cells gene expression. Genetic inactivation of WNT signaling reversed age-dependent changes of mTORC1-driven lung phenotype, but WNT activation alone in lung mesenchyme was not sufficient for the development of mouse LAM-like phenotype. The alterations in gene expression were driven by distinctive crosstalk between mesenchymal and epithelial subsets of cells observed in mesenchymal Tsc2-deficient lungs. In conclusion, we created a mouse model with lung-specific Tsc2 KO which recapitulates many aspects of human pulmonary LAM, identified sex- and age-specific gene changes in the mTORC1-activated lung mesenchyme, and established the importance of the WNT signaling pathway in the mTORC1-driven lung phenotype.

3. Differential Role of Skeletal Muscle AKT Signaling in the Regulation of Glucose Metabolism and Muscle Growth

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Skeletal muscle is the predominant site of insulin-mediated glucose uptake in the postprandial state, a reduction in the insulin signaling pathway of diabetic skeletal muscle is widely considered to be the primary cause of postprandial hyperglycemia. The serine/threonine kinase AKT is a central regulator of insulin action and a decrease in AKT activity is observed in muscle from insulin-resistant mice and humans. This has understandably led to the dogma that impaired AKT activity in skeletal muscle causes defects in glucose homeostasis and muscle function. To directly test this hypothesis, we generated several mouse models of skeletal muscle AKT deficiency. Unexpectedly, mice lacking AKT2 alone, exhibited normal insulin signaling, insulin sensitivity and muscle mass despite a dramatic reduction in phosphorylated muscle AKT. In contrast, deletion of both muscle AKT isoforms (M-AKTDKO) resulted in a complete loss of AKT-mediated insulin signaling. Despite the lack of AKT activity, M-AKTDKO mice were insulin sensitive. This chronic loss of AKT was associated with mitochondrial dysfunction and subsequent AMPK activation, which we identified to be an important regulator of muscle insulin sensitivity. Unlike glucose metabolism, loss of muscle AKT resulted in muscle atrophy and defective muscle performance. Mechanistically, activation of mTORC1 and inhibition FOXO1 were both required and sufficient to induce muscle hypertrophy in the absence of AKT. Thus, while AKT signaling is required for muscle growth, AKT is not an obligate intermediate for insulin-stimulated glucose uptake in all conditions. Collectively, these data suggest the existence of additional insulin-dependent, AKT-independent signaling pathways for the regulation of glucose homeostasis.

4. Exercise training rescues exercise tolerance and attenuates features of aging in skeletal muscle of Bmal1 KO mice

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The mammalian circadian clock orchestrates rhythmic gene expression and regulates biological functions through the master genes Bmal1 (Arntl) and Clock. Bmal1-/- knockout (KO) mice exhibit a phenotype of accelerated ageing including an age-dependent reduction of muscle mass and alterations to muscle physiology. Considering exercise is associated with attenuating the effects of sarcopenia in humans, we hypothesized that exercise may ameliorate some of the negative consequences of Bmal1 KO in mice. Therefore, 8-week old wild-type (WT) and Bmal1 KO mice were subjected to endurance exercise training on a treadmill for 3 times/week at ZT 0-4 for 50 min (17 m/min) for 8 weeks. Following 8 weeks of training, the mice were subjected to an exercise tolerance test of increasing speed and incline until the mice reached exhaustion. After sacrifice at 18 weeks, the soleus muscle was dissected, sectioned, and analyzed using hematoxylin and eosin (H&E) staining and immunofluorescence fiber typing. In sedentary mice, exercise tolerance is significantly reduced in Bmal1 KO mice, whereas WT and KO mice performed similarly following endurance training. Analysis and scoring of H&E stained soleus tissue demonstrated that sedentary KO mice had higher levels of fibrosis and central nuclei (indicative of regeneration) than WT. Notably, exercise significantly increased central nuclei levels in both WT and KO mice. Immunofluorescence fiber typing showed that sedentary Bmal1 KO mice have fewer type I than type IIa fibers, which is reversed as result of exercise, potentially indicating increased muscle endurance. In conclusion, endurance exercise rescues the negative effects of Bmal1 deficiency on exercise tolerance, which may be linked to physiological changes in muscle fibers.

5. Novel algorithm accurately detects errors in growth data using Gaussian Process regression and Bayesian outlier methods

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Body mass index (BMI) is a straightforward and powerful metric in obesity and other growth-related studies. Unfortunately, it is not always straightforward to identify and remove spurious measurements due to unit errors, digit transpositions and other transcription errors. In many cases, it is not clear what constitutes a realistic change in BMI, and what constitutes an erroneous measurement. This is particularly true in pediatric cohorts, in which patients' BMIs are naturally changing as a function of their development. We have developed an algorithm based on Gaussian Process Regression and Bayesian outlier detection methods that can flexibly fit noisy, highly variable BMI data and reject unrealistic BMI measurements. The Bayesian outlier detection is performed on the height data, which can be fit parametrically, and provides a statistically robust estimate of the probability that a given point is an outlier. The Gaussian Process performs a flexible, non-parametric fit to the data that can account for naturally occurring fluctuations in weight. Our algorithm has been used to select obese pediatric patients for a large-scale sequencing effort with approximately 99% success - that is, patients with erroneously high BMI measures were properly excluded. Work is underway to deploy this algorithm across both pediatric and adult sites in the Electronic Medical Records and Genomics (eMERGE) consortium, a multi-site collaboration that is creating robust phenotyping and polygenic risk score algorithms for multiple phenotypes. Many of these algorithms depend upon reliably cleaned and accurate BMI measurements.

6. Targeting androgen regulation of TMPRSS2 and ACE2 as a therapeutic strategy to combat COVID-19

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The COVID-19 pandemic is expected to have an adverse effect on the progression of multiple cancers, including prostate cancer, due to the ensuing cytokine storm and associated oncogenic signaling. Epidemiological data showing increased severity and mortality of COVID-19 in men suggests a potential role for androgen in SARS-CoV-2 infection. Here, we present evidence for the transcriptional regulation of SARS-CoV-2 host cell receptor ACE2 and co-receptor TMPRSS2 by androgen in mouse tissues and human prostate and lung cell lines. Additionally, we demonstrate the endogenous interaction between TMPRSS2 and ACE2 in human cells and validate ACE2 as a TMPRSS2 substrate. In an overexpression model, and in the prostate and lung cells, Camostat (a TMPRSS2 inhibitor) blocked the cleavage of pseudotype SARS-CoV-2 surface Spike. In these models, TMPRSS2-ACE2 interaction was not disrupted thus providing evidence for a direct role of TMPRSS2 in priming the SARS-CoV-2 Spike protein, required for viral fusion to the host cell. Importantly, androgen-deprivation, anti-androgens such as enzalutamide/AR-PROTAC, or Camostat treatment attenuated the SARS-CoV-2 S-mediated entry in lung and prostate cells. Together, our preclinical data provide a strong rationale for clinical evaluations of the TMPRSS2 inhibitors, androgen-deprivation therapy, and androgen receptor antagonists alone or in combination with anti-viral drugs as early as clinically possible to prevent inflammation driven COVID-19 progression.

7. HIV pre-exposure prophylaxis counseling among non-Hispanic Black youth diagnosed with bacterial STIs, 2014-2019

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Youth account for a disproportionate number of new HIV infections; however, PrEP use among youth is limited. We evaluated rates of PrEP counseling among Black youth after an incident bacterial STI diagnosis. We conducted a retrospective cohort study of non-Hispanic Black youth at two clinics in Philadelphia who met 2017 CDC eligibility criteria for PrEP due to qualifying bacterial STI between June 2014 and June 2019. We compared PrEP counseling rates for youth who received primary care services versus those who did not receive primary care services. Among our cohort of 416 PrEP-eligible youth, 35 patients (8%) had documentation of PrEP counseling. Receipt of primary care services was not significantly associated with receipt of PrEP counseling after adjusting for patient- and health care-level factors (aOR 0.30 (95% CI 0.05 to 1.84)). Being assigned male sex at birth (aOR = 40.2, 95% CI 3.32 to 487) and having a rectal STI (aOR = 61.7, 95% CI 6.63 to 574) were strongly associated with receipt of PrEP counseling. We found that PrEP-eligible non-Hispanic Black youth receive PrEP counseling at low rates, and recent bacterial STI diagnosis is a frequently missed opportunity for medical providers to inform and counsel young Black patients assigned female sex at birth about PrEP. These findings support the need for robust investment in PrEP-inclusive sexual health services that are widely implemented and culturally tailored to Black youth at risk of HIV acquisition, particularly cisgender heterosexual females.

8. Dolutegravir Inhibits Oligodendrocyte Maturation: Roles for The Integrated Stress Response and Lysosomal Homeostasis

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Thanks to the development of antiretroviral therapies (ART), HIV-1 infection has shifted from a fatal disease to a chronic, yet clinically manageable disorder. However, vertical HIV-1 transmission persists in geographic locations where ART availability is limited. As a result, the numbers of perinatally HIV-1 infected (PHIV) children and perinatally HIV-1 exposed, but unaffected (PHEU) children continue to increase in the clinical population. Recent DTI and MRI imaging studies on PHIV children showed dramatic thinning of the major white matter tracks in their brains, which may contribute to their impaired neurodevelopmental milestones as they age, even while adhering to ART. These findings led us to hypothesize that in utero exposure to specific ART drugs lead to impaired white matter development though dysregulated differentiation of oligodendrocyte precursor cells (OPCs) into mature oligodendrocytes. In this current study, we utilized an ART regimen which is commonly prescribed to pregnant HIV-1 infected women: Abacavir, Lamivudine, and Dolutegravir. Using a well-characterized in vitro OPC culture system, we demonstrate that Dolutegravir inhibits oligodendrocyte differentiation and myelin protein production in a concentration-dependent manner. However, Abacavir and Lamivudine do not affect oligodendrocyte differentiation and myelination. Additionally, treatment of Dolutegravir-treated oligodendrocyte precursor cells with two drugs, one which dampens the integrated stress response and the other re-acidifies endolysosomes rescue low, but not mid dose differentiation deficits. Together this data demonstrates a potential role for the integrated stress response and lysosomal physiology in Dolutegravir-induced oligodendrocyte differentiation deficits.

9. The Cryptosporidium single-cell atlas reveals key life cycle stages and a commitment to male and female development

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The apicomplexan parasite Cryptosporidium is a leading global cause of diarrheal disease and infects millions of people each year. With no vaccine and inadequate treatment, a great need exists for new and more effective therapeutics. Transmission occurs via the fecal-oral route, and the entire parasite life cycle takes place in a single host: asexual growth takes place in intestinal epithelial cells, followed by transition to a male or female form and sexual reproduction. Because gene expression changes associated with parasite development remain largely unknown, we used single-cell RNA sequencing of infected cultures and mice to determine the complete life cycle transcriptome of Cryptosporidium in vitro and in vivo. Analysis of 9,310 individual parasite transcriptomes revealed clear asexual cycle progression with a direct switch to either male or female development during the trophozoite stage, with no evidence for a prior sexual commitment stage. In asexual parasites, gene expression was driven by cell cycle progression dominated early by ribosomal biogenesis, processing, and assembly followed by protein folding and DNA replication. Later asexual stages expressed many secreted proteins, including invasion related organelles. Importantly, single-cell transcriptomics revealed stage-specific and sex-specific expression of key transcription factors, including a Myb gene only expressed in the earliest males, outlining a pathway for sex-specific commitment. Future work will focus on determining the functional roles of these regulators. Overall, our work provides the first comprehensive view of Cryptosporidium gene expression over the entire life cycle and identifies the key genes in replicative, invasive, and sexual stages and the regulatory networks that control them.

Posters

Structure of the ancestral TRPY1 channel from *Saccharomyces cerevisiae* reveals mechanisms of lipid and calcium modulation

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Transient Receptor Potential (TRP) channels have evolved in mammals and other higher organisms to control various cellular functions in response to a wide variety of chemical and physical stimuli. This large and diverse family of eukaryotic channels first emerged in fungi where they are mainly responsible for osmoregulation and are considered to be mechanosensitive. The *Saccharomyces cerevisiae* vacuolar transient receptor potential yeast 1 (TRPY1) is the most studied TRP channel from fungi, but the molecular details of channel modulation remain elusive so far. Here, we describe the full-length cryo-electron microscope (cryo-EM) structure of TRPY1 at 3.1 Å resolution. The structure reveals a unique architecture for TRPY1 among all eukaryotic TRP channels with an evolutionarily conserved and archetypal transmembrane domain, but distinct structural folds formed by cytoplasmic N- and C-termini. We identified the inhibitory phosphatidylinositol 3-phosphate (PI(3)P) lipid binding site, which sheds light into the lipid modulation of TRPY1 in vacuolar membrane. We also elucidated two Ca^{2+} binding sites: one in the cytoplasmic side, implicated in activation and the other in the vacuolar lumen side, involved in channel inhibition. These findings together with data from molecular dynamics simulations provide structural insights into understanding the basis of TRPY1 channel modulation by Ca^{2+} and lipids.

The Role of CTCF in the Organization of the Centromeric 11p15 Imprinted Domain Interactome

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Loss of methylation at the human centromeric KCNQ1OT1 Imprinting Center 2 (IC2) is the most common cause of Beckwith-Wiedemann Syndrome (BWS). Here, we report a familial transmission of a 6.8 kB deletion at the 5' end of KCNQ1 within this domain that causes BWS due to loss of methylation at IC2 in addition to both perturbation of the transcription of the KCNQ1 gene and the surrounding chromatin architecture. Importantly, the abrogated maternal CTCF binding caused chromatin architecture changes in cis. Based on these results, we propose a model of IC2 imprinting regulated by CTCF-mediated chromatin looping, wherein KCNQ1 and CDKN1C transcription is driven by their physical association on the maternal allele that facilitates an enhancer interaction.

Defining the role of pulmonary endothelial cells in regeneration of the lung

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To facilitate gas exchange between the lungs and the cardiovascular system, the lung possesses the largest organ-specific vascular network in mammals. When the lung is injured, it is essential to rebuild the gas exchange machinery, including the key interface between pulmonary endothelial cells (ECs) and type I alveolar epithelial cells. Although ECs represent one half of the gas exchange interface, their role in regeneration is unclear, presenting a barrier to improving functional regeneration. To test the hypothesis that EC subtypes in the lung may be specialized to act as endothelial progenitors or signaling hubs after injury, we profiled mouse lung EC heterogeneity at single-cell resolution using scRNA-seq. We then investigated each unique EC population at homeostasis and after influenza injury using flow cytometry, in situ hybridization, and immunohistochemistry. We determined that although EC proliferation increases dramatically following acute lung injury, no one population of ECs serves as a progenitor cell; rather, multiple pulmonary EC populations possess the capacity to proliferate. We also identified a population of Car4-high ECs that express high levels of signaling molecules and localize within sites of moderate-to-severe alveolar damage after influenza infection, suggesting that their signaling capacity may be essential to lung repair. To further define the importance of pulmonary EC subtypes, we have used mouse genetics to specifically trace several EC populations at homeostasis and after injury and determine their individual contributions to alveolar regeneration. These studies will facilitate a better understanding of how the lung vasculature is rebuilt to reestablish gas exchange during lung regeneration.

Six weeks of chronic partial sleep restriction in a spaceflight analog induce deficits in cognitive performance

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Space exploration missions to the Moon and Mars will challenge crews with never-before-seen physical and cognitive demands in increasingly autonomous operations. Modeling the heavy workload and recovery sleep cycle pattern often observed aboard the International Space Station, we assessed the effects of 6 weeks of partial chronic sleep restriction on cognitive performance in 15 high-performing individuals (38.5 3 8.2 years, 6 women) confined to NASA's Human Exploration Research Analog habitat in groups of 4. Sleep opportunities were limited to 5h per night Monday through Friday with 8h recovery opportunities on weekend nights. Individual sleep/wake patterns were measured continuously with actigraphy. Cognitive performance was assessed with a computerized battery of ten brief tests assessing a range of cognitive domains including working memory, sensorimotor speed, risk taking, and vigilant attention. Subjective measures of sleep and wellbeing were also measured. With increasing sleep debt relative to mean pre-mission baseline, there were decreases in accuracy across cognitive domains ($p<0.001$), specifically on tests of spatial orientation ($p=0.020$) and vigilant attention ($p<0.001$), as well as lower subjective ratings of happiness ($p<0.001$) and healthiness ($p<0.001$) and higher subjective stress ($p=0.036$). Cognitive performance decrements were not restored by two nights of weekend recovery sleep. With decreasing total sleep time in the prior sleep period, subjects reported increasing levels of sleepiness, physical exhaustion, mental fatigue, tiredness and monotony. Collectively, these findings underline the importance of sufficient sleep, both on an acute and a chronic basis, for cognitive performance and subjective wellbeing in operationally-relevant environments.

Manipulation of Tropomyosin 1 in iPSCs to enhance in vitro blood cell production

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Donor-derived blood transfusions are critical to our healthcare system, but do not fully meet the needs of patients with multiple alloantibodies, rare blood types, or HLA-sensitization. In vitro-derived blood cells could mitigate these issues, but inefficient in vitro cell yields pose a major challenge. We previously used machine learning and genome-edited induced pluripotent stem cell (iPSC) models to determine that Tropomyosin 1 (TPM1) normally inhibits in vitro hematopoiesis. TPM1 knockout (TPM1KO) iPSCs produced 2-fold more hematopoietic progenitor cells (HPCs) than controls, thereby increasing yields of mature blood cells that were functionally normal. During human hematopoiesis, HPCs arise from specialized vascular 'hemogenic endothelial' cells (HE). RNA sequencing analysis of sorted KDR+CD31+ endothelial cells and CD43+ HPCs from TPM1KO and controls suggested that TPM1KO increased HE production and/or HPC survival. TPM1KO endothelial cells and HPCs had altered expression of genes and pathways known to regulate HE biology, including cell adhesion, integrin expression, and integrin-mediated signaling ($p<0.05$). Indeed, in limiting dilution analyses, TPM1KO cultures produced 2-fold more HE than controls. Further, TPM1KO endothelial cells had increased expression of N-cadherin and RAP1-activating genes. Such changes limit 'anoikis', an apoptosis-like process that can occur after extracellular matrix detachment, and may promote nascent HPC survival. These results show that TPM1KO enhances in vitro hematopoiesis by increasing HE and subsequent HPC production. Genetic or pharmacologic manipulation of these novel mechanisms will help boost in vitro HPC and blood cell production to clinically relevant scale.

Effectiveness and safety of direct oral anticoagulants versus warfarin in patients with valvular atrial fibrillation

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The significance of this study is that with no requirement for frequent monitoring and fewer drug interactions, direct-oral anticoagulants (DOACs) are increasingly used in place of warfarin, but evidence regarding their effectiveness and safety in patients with valvular atrial fibrillation (VAF) remains limited.

Our objective is to assess the effectiveness and safety of DOACs compared to warfarin in patients with VAF. To do this we use a new-user retrospective propensity score-matched cohort study with adults with VAF including aortic, mitral, tricuspid or pulmonary valve disease as participants. Exposure of participants included initiation DOACs or warfarin between January 2010 and June 2019.

The primary effectiveness outcome was a composite of ischemic stroke [IS] or systemic embolism [SE]. The primary safety outcome was major bleeding events. We controlled for measured confounders via propensity-score matching and used Cox proportional hazard regression with a robust variance estimator to generate marginal hazard ratios (HRs). We examined the within-class effect of the individual DOACs compared to warfarin. Among a total of 56,336 propensity score-matched persons with VAF, use of DOACs (vs. warfarin) was associated with a lower risk of IS/SE (HR=0.64, 0.59–0.70) and major bleeding events (HR=0.67, 0.63–0.72). Results remained consistent for IS/SE and bleeding with apixaban (HR=0.54, 0.47–0.61 and HR=0.52, 0.47–0.57) and rivaroxaban (HR=0.74, 0.64–0.86 and HR=0.87, 0.79–0.96). In this comparative effectiveness study, persons with VAF who were new-users of DOACs had a lower risk of IS or SE and major bleeding compared with warfarin. In the absence of head-to-head prospective studies, these data may be used to guide risk-benefit discussions regarding anticoagulant choices for patients with VAF.

Oncogene-driven de novo enhancer assembly promotes malignancy in Ewing sarcoma via aberrant expression of LOXHD1

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Ewing sarcoma is a highly aggressive tumor of bone and soft tissues that mostly affects children and adolescents. The oncogenic transcription program of Ewing sarcoma is orchestrated by the pathognomonic fusion EWS/ETS (EWS-FLI1/EWS-ERG) transcription factors. Pharmacological targeting of this oncogenic fusion transcription factor has been challenged by the presence of unstructured prion-like domains in the EWSR1 portion of the fusion. Alternatively, identification and characterization of mediators and downstream targets of EWS/ETS dependent/independent function could offer novel therapeutic options. By sequence data mining of in-house and publicly available databases which comprise more than 20,000 genomic and transcriptomic datasets representing primary cancer, metastasis and normal tissues, we identified LOXHD1 as a gene uniquely expressed in Ewing Sarcoma. Integrative analysis of genome wide FLI1 occupancy and multiple histone mark enrichment patterns in Ewing Sarcoma cell lines showed that LOXHD1 has a unique gene structure in Ewing sarcoma cells compared to the current RefSeq annotations and is regulated by EWS-ETS binding to an upstream GGAA de novo enhancer. We confirmed the results with multiple genetic editing assays. We performed functional studies with in vitro cell lines and in vivo animal and embryo models and established the role of LOXHD1 in regulating EWS tumor growth and metastasis. We found LOXHD1 as a major determinant of Ewing sarcoma development through regulating cytoskeleton homeostasis and hypoxic stress response by chaperoning HIF1a stability. Finally, our discovery that LOXHD1 is expressed in a highly specific manner in human Ewing sarcoma opens up new avenues for developing LOXHD1-targeted cellular therapies in the future.

Sleep duration and alertness among internal medicine interns comparing intensive care unit to general medicine rotations

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We examine differences in sleep duration and alertness among internal-medicine interns during intensive care unit (ICU) compared to general medicine (GM) rotations. This is a secondary report of a randomized non-inferiority trial of 63 US internal-medicine residency programs assigned to either standard duty-hour (80h workweek/16h shifts) or flexible (80h workweek/no shift-length limit) policies. Interns were followed for 2 weeks during either GM or ICU rotations. Primary outcome was sleep duration/24h (actigraphy). Secondary outcomes were sleepiness (Karolinska Sleepiness Scale [KSS]) and alertness (number of Brief Psychomotor Vigilance Test [PVT-B] lapses). Data were averaged across days (13 24-hour periods). Linear mixed-effect models with random program intercept were used to determine association between each outcome by rotation, controlling for age, sex, and policy followed. N=386 interns were included (mean age 27.932.1y, 194 (50.3%) males, n=261 (67.6%) GM, and n=125 (32.4%) ICU). Mean sleep duration was 7.0030.08h and 6.8430.10h for GM and ICU respectively (p=.09; 95%CI -0.02; 0.33h). Percent of days with reports of excessive sleepiness were significantly more likely for ICU vs GM from 12am-6am (ICU: 20.2%; GM: 12.5%) and 6am-12pm (ICU: 20.5%; GM: 14.3%). GM had significantly more days with no excessive sleepiness (GM: 40.5%; ICU: 28.1%). Average KSS was 4.830.1 for both GM and ICU (p=.60; 95%CI -0.18; 0.32). Average number of PVT-B lapses were 5.530.5 and 5.730.7 for GM and ICU respectively (p=.83; 95%CI -1.48; 1.18 lapses). Interns in ICU experienced more excessive sleepiness compared to GM interns, especially in early morning hours. However, sleep duration and alertness were not significantly different between rotations.

Essential roles of Akt-dependent regulation of nitric oxide synthase and inflammation in cystic fibrosis (CF)

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Protease-activated receptor (PAR-2) plays a role in inflammation in the airway. Cystic fibrosis (CF) is a lung disease in which cells may be hyperinflammatory with increased levels of cytokines, such as interleukin (IL)-8. Activation of the Akt/endothelial nitric oxide synthase (eNOS) pathway is dysregulated in a variety of diseases, possibly including CF. We hypothesized that the aberrant regulation of p-Akt, p-eNOS, and inflammation may occur in CF cells during PAR-2 stimulation. To test if phosphorylated (p)-Akt levels are disrupted by CFTR mutations, Wild-type (Wt), and CFTR mutant (ΔF508 and G542X) 16HBE cells were lysed for qPCR and Western. To determine if CFTR mutations affected p-Akt and p-enos protein levels during PAR-2 stimulation, Wt and mutant cells were stimulated with 2-FLI (20 uM) for 5 min before harvesting for Western blotting. To test if PAR-2 stimulation upregulates IL-8, Wt and mutant cells were treated with 2-FLI for 6 hrs before lysing for qPCR for IL-8. We observed equal mRNA expressions for all three isoforms of Akt in ΔF508, G542X, and Wt 16HBE cells. However, p-Akt (S473) levels in G542X cells are reduced significantly ($p<0.01$) compared to ΔF508 and Wt cells. Further, both p-Akt and p-eNOS levels were significantly elevated ($p<0.05$) in Wt cells compared to mutant cells. Additionally, stimulation of PAR-2 with 2- FLI significantly increased IL-8 in CFTR mutant cells compared to Wt cells. Our preliminary data suggest that dysregulation of Akt/eNOS may contribute to the increase in inflammatory cytokines such as IL-8. Future work will be directed towards testing some of the above studies in primary upper airway cells from CF patients to validate our results.

Crosstalk between Notch signaling and cancer-associated fibroblasts drive radio-resistance in aggressive breast cancer

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Resistance to radiotherapy remains a major obstacle in modern cancer therapy. Several lines of recent evidence support aberrant Notch signaling in tumor cells is well associated with therapeutic resistances in breast cancer. However, therapeutic failure is not only dictated by events involving only tumor cells, but also by the tumor microenvironment (TME). Therefore, a stronger understanding of the intricate interplay between tumor cells and cancer-associated fibroblasts (CAFs), one of crucial component of TME in acquiring radio-resistance are desperately needed to improve outcomes for breast cancer patients. In this study, we showed Notch ligand Dll1 drive radio-resistance in aggressive breast cancer using Dll1 conditional-knockout (Dll1cKO) and Dll1mCherry reporter mouse models in MMTV-PyMT breast cancer background. To identify possible crosstalk between Dll1+ tumor cells and breast TME, CAFs from irradiated breast tumor cells were isolated and characterized using novel flowcytometric approach. We moreover showed, with post radiation exposure, enhanced abundance of CAFs within tumor cells promotes hypoxia, and CAF-educated tumor cells promote stemness activity. Our further in vitro studies revealed that Dll1+ tumor cells are responsible for recruitment of CAFs by cytokine (IL-6, IL-12) and chemokine (CXCL-12) response in radio-resistant breast TME. Ongoing work is focused on RNA seq approach to identify factors secreted by CAFs, supporting stem cell function of tumor cells. Overall, our findings will generate a strong foundation for therapeutic applications aimed to promote radio-resistance recovery in breast cancer patients.

Paradoxical function of Natural Killer cells in triple negative breast cancer

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Triple Negative Breast Cancer (TNBC) is the most aggressive type of breast cancer, with limited therapeutic options and high mortality. While the high level of immune infiltrate in TNBCs suggested that they would be amenable to targeted immunotherapy, phase-I clinical trials with PDL-1 inhibitors have shown very limited responses. Thus, additional understanding of the immune landscape in the TME of TNBC patients is representing a clinically unmet need. Natural Killer (NK) cells are cytotoxic lymphocytes, classically known to act against tumor cells. However, preliminary studies with our novel TNBC mouse model showed NK cells that are source of IFN-γ were surprisingly overrepresented in aggressive TNBCs and raised an intriguing possibility of activating IFN-γ signaling therefore upregulating IFN-γ responsive target genes such as PDL-1 to contribute to the aggressive and metastatic nature of TNBC. We have observed distinct subsets of NK cells in aggressive TNBCs through scRNA seq that express high levels of IFN-γ and low levels of cytotoxic granzymes. Both, in silico analysis and high CD56 expression in patient tumor tissues revealed that NK cells are significantly correlated with poor Overall Survival (OS) in TNBC patients. Pharmacological blocking of NK cells either alone or in combination with PD-L1 in tumor bearing mice reduced tumor burden and metastasis. Moreover, NK cells isolated from TNBC tumors showed less cytotoxicity and were protumorigenic when co-cultured with TNBC cells supported the paradoxical function of these cells. This work unravels the function and identity of protumorigenic NK cell subsets that may be used as a prognostic factor and can be therapeutically targeted to improve the outcome for TNBC patients.

Dissecting phenotypic transitions in metastatic disease via photoconversion-based isolation

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Cancer patients presenting with surgically resectable disease often harbor occult metastases, a potential source of relapse that is targetable only through systemic therapy. Studies of this occult fraction have been limited by a lack of tools with which to isolate discrete cells based on spatial grounds. We developed PIC-IT, photoconversion-based isolation technique allowing efficient recovery of cell clusters of any size including solitary disseminated tumor cells (DTCs), which are largely inaccessible otherwise. In a murine pancreatic cancer model, transcriptional profiling of spontaneously arising DTCs revealed phenotypic heterogeneity, functionally reduced propensity to proliferate and enrichment for inflammatory-response phenotype associated with NF-κB /AP-1 signaling. Pharmacological inhibition of NF-κB depleted DTCs but had no effect on macrometastases, suggesting DTCs are particularly dependent on this pathway. PIC-IT enables systematic investigation of the earliest stages of metastatic colonization. Moreover, this new technique can be applied to other biological systems in which isolation and characterization of spatially distinct cell populations is not currently feasible.

Rescue of social isolation induced death in ants

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Social isolation is likely to increase as a function of old age, depression, and as a way to combat global pandemics. It remains unknown how social isolation impacts physiology, lifespan, genetic and epigenetic regulation in a social organism. In this study, we demonstrate that social isolation of the ant species *Camponotus floridanus* results in a dramatically shortened lifespan, changes in brain morphology and locomotion, accompanied by genetic and epigenetic changes. We are able to rescue these negative phenotypic effects through the use of 3D printed ants coated with ant cuticular hydrocarbons, suggesting that social perception and interaction are critical to brain development and lifespan. These social instances are mediated, in part, by olfactory, visual, and tactile cues. Furthermore, we identify the gene Cylindromatosis (CYLD), which increases expression in isolated ants, but not in group housed and rescue conditions. Brain specific knockdown of CYLD extends lifespan of isolated ants, providing the first genetic and mechanistic link between social interaction and lifespan. Our findings provide the foundational basis of social regulation of lifespan, genetics, epigenetics, and behavior in ants. Furthermore, we demonstrate the unique ability to rescue phenotypic changes through the use of surrogate 3D printed ants and genetic manipulation.

Baseline inflammatory cytokine and cortisol levels predict mood and working memory deficits induced by sleep restriction

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The inter-individual differences in the neurobehavioral responses to sleep restriction are pronounced. Predictors of vulnerability to sleep restriction have included demographic, genetic, and metabolic factors, however whether inflammatory cytokine or cortisol levels are involved is unknown, despite being promising targets due to established roles in sleep regulation and neurobehavioral function N=124 healthy participants (52% female; n=64), 22-45 years of age, completed two baseline nights (8h time in bed [TIB]) followed by five nights of 4h TIB. IL-6, TNF-α, and cortisol levels were measured in venous blood on the second baseline day. Subjective mood state (profile of mood states [POMS]) and working memory (digit span test [DST]) were assessed every 2h during wakefulness. Mixed-effects models, adjusted for baseline, age, and sex, evaluated the relationships between IL-6, TNF-α, and cortisol levels and the POMS and DST, independently. At baseline, IL-6, TNF-α, and cortisol levels were not associated with POMS or DST. There was a main effect of IL-6, but not cortisol or TNF-α, levels on mood disturbance (β=3.060; P=0.0041). Higher cortisol levels predicted increasing mood disturbance across days (β=-0.838; P=0.0004). Higher TNF-α levels predicted degrading DST performance across days (β=-0.470; P=0.0007). Higher IL-6 (β=-0.232; P=0.017) and lower cortisol (β=-0.232; P=0.035) levels also predicted degrading DST performance across days. The study findings suggest that basal inflammatory cytokine and cortisol levels are implicated in the individual risk of mood disturbance and working memory deficits resulting from sleep restriction and highlight the need to consider biological processes and phenotypes together.

Plasma-derived extracellular vesicles induced STING-mediated proinflammatory responses in dermatomyositis

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Dermatomyositis (DM) is an acquired inflammatory myopathy characterized by chronic skin inflammation. Extracellular vesicles (EVs) are lipid bilayer membrane vesicles existing in various bodily fluids and implicated in the pathogenesis of autoimmune diseases. As type I interferons, specifically IFN- are uniquely elevated in DM, and Stimulator of interferon genes (STING) works as a critical sensor and adaptor in type I IFN signaling, we hypothesized that EVs derived from DM plasma might trigger STING-mediated proinflammatory effects. DM patients were recruited in the PennDerm. PBMCs were isolated by Ficoll gradient. EVs derived from plasma were isolated via ultracentrifugation. The supernatant was harvested for ELISA and the lysed cells were collected for Western blot. DM plasma derived EVs triggered more cytokines release than HC with STING phosphorylation in PBMCs. Inhibition of STING significantly attenuated DM plasma derived EVs-triggered cytokines production via suppressing STING signaling pathway phosphorylation. Besides, TBK1 inhibitors also suppressed DM plasma derived EVs-induced IFNb release by inhibiting TBK1 phosphorylation. To further explore whether STING mediated proinflammatory effects were caused by EVs-captured dsDNA, EVs were pretreated with Triton X-100 and DNase to digest DNA. Triton X-100 and DNase pretreatment decreased EVs-triggered cytokines release and STING activation. EVs derived from plasma could trigger STING-mediated proinflammatory effects in DM. The STING signaling pathway activation during EVs triggering of proinflammatory effects was at least partially mediated by dsDNA captured by EVs. Targeting STING pathway might provide insight into a potential therapeutic approach for DM.

Biophysical quantification of cross-kingdom interactions between streptococci and different morphotypes of *C. albicans*

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Candida albicans is a frequently detected fungal pathogen in the oral cavity. It predominantly exists in two morphotypes, yeast cells and hyphae, and forms polymicrobial biofilms with several bacterial species. Specifically, its synergistic interactions with streptococci lead to biofilms with greater microbial carriage, infectivity, and antimicrobial resistance. These interactions vary in mechanism. For example, *Streptococcus gordonii* uses direct cell-cell interactions to bind avidly to *C. albicans* hyphae while *Streptococcus mutans* interacts with *C. albicans* via extracellular glucans. However, at the single-cell level, the biophysical aspects of such synergistic cross-kingdom interactions that aid biofilm initiation need further investigation. Here, we measured the binding forces between *C. albicans* (as yeast cells and hyphae) and *S. mutans* (or *S. gordonii*) in the presence and absence of in situ glucans on the fungal surface using single-cell atomic force microscopy. Our data show that *S. gordonii* preferred to bind to hyphal *C. albicans* rather than yeast cells (~2.5-fold increase). The presence of in situ glucans lowered these binding forces in comparison to uncoated *C. albicans* indicating an obstruction of direct cell-cell interaction. On the other hand, *S. mutans* displayed similar preference for binding to *C. albicans* as yeast cells or hyphae. The presence of in situ glucans dramatically enhanced the binding forces in comparison to uncoated *C. albicans* (up to ~6-fold increase). This study provides a novel biophysical aspect to elucidate *C. albicans*-streptococcal interactions in terms of fungal morphotype and extracellular glucans. Our results can lead to an improved understanding of the early microbial alliances involved in cariogenic biofilm development.

Therapeutic targeting of TMPRSS2 and ACE2 as a potential strategy to combat COVID-19

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The SARS-CoV-2 infection responsible for the COVID-19 pandemic is expected to have an adverse effect on the progression of multiple cancers, including prostate cancer, due to the ensuing cytokine storm associated oncogenic signaling. Epidemiological data showing increased severity and mortality of COVID-19 in men suggest a potential role for androgen in SARS-CoV-2 infection and host response. Here, we present evidence for the transcriptional regulation of SARS-CoV-2 host cell co-receptor TMPRSS2 and receptor ACE2 by androgen in mouse tissues, human prostate, and lung cells. Additionally, we demonstrate the endogenous interaction between TMPRSS2 and ACE2 in human cells and validate ACE2 as a TMPRSS2 substrate. In the prostate and lung cells, camostat – a serine-protease inhibitor specific to TMPRSS2 - inhibited the cleavage of pseudotype SARS-CoV-2 surface Spike without disrupting TMPRSS2-ACE2 interaction. Thus, providing evidence for the first time a direct role of TMPRSS2 in priming the SARS-CoV-2 Spike protein, required for viral fusion to the host cell. Importantly, androgen-deprivation, anti-androgens such as enzalutamide/AR-PROTAC, or camostat treatment attenuated the SARS-CoV-2 pseudotype entry in the lung and prostate cells. Together, our preclinical data provide a strong rationale for clinical evaluations of the TMPRSS2 inhibitors, androgen-deprivation therapy, and AR-signaling blockers alone or in combination with anti-viral drugs as early as clinically possible to prevent inflammation driven COVID-19 progression in men with or prostate without cancer.

Single-cell transcriptomics and cell-specific proteomics reveals molecular signatures of sleep

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Sleep is an essential component of daily life. The explicit function of sleep is still a mystery. Insufficient or disturbed sleep is associated with the accumulation of brain waste, cognitive impairments, increased risk of metabolic abnormalities, and suppressed immune responses. Restoration of many of these abnormalities by sleep indicates sleep loss is associated with perturbation of cellular and molecular functions. Homeostatic regulation of sleep is maintained by progressively rising sleep need during wakefulness, which then dissipates during sleep. The molecular mechanisms governing sleep are largely unknown. Here, we used a combination of single cell RNA sequencing and cell-type specific proteomics to interrogate the molecular underpinnings of sleep. We performed EEG/EMG study to validate our sleep model. Further, we collected the brainstem, cortex and hypothalamus from normal, sleep deprived (12 h) and recovery sleep (24 h) mice to prepare cell-suspensions for single cell RNA sequencing. We separated astrocytes and neurons from cortical single cell suspensions and performed cell-specific proteomics. Different cell types in three important brain regions for sleep (brainstem, cortex and hypothalamus) exhibited diverse transcriptional responses to sleep need. Sleep restriction modulates astrocyte-neuron crosstalk and sleep need enhances expression of specific sets of transcription factors in different brain regions. In cortex, we also interrogated the proteome of two major cell types: astrocytes and neurons. Sleep deprivation differentially alters the expression of proteins in astrocytes and neurons. Similarly, phosphoproteomics revealed large shifts in cell-type specific protein phosphorylation. Our results indicate that sleep need regulates transcriptional, translational and post-translational responses in a cell-specific manner.

Measurement and control of the proteome through scalable tagging

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Since deciphering the human genetic code, a major challenge in biology has been to understand the functions of all the proteins encoded by our genes. Traditionally, however, the study of proteins has largely relied on biochemical and genetic tools that are best suited for analyzing one protein at a time and are thus too costly and laborious for studying proteins at scale. A strategy for high-throughput manipulation and analysis of individual proteins would consequently enable large and novel insights into the human proteome. Here, we describe a scalable system in which we can change individual protein levels, folding states, and fluorescent properties, and subsequently detect the effects of these changes on the protein itself as well as on the larger cellular environment. This strategy involves tagging all proteins with a manipulable protein domain by homology-independent intron targeting. Only one protein is tagged per cell, and a pool of differentially-tagged cells represent the entire proteome. This pool can be manipulated and then analyzed by deep sequencing, fluorescent imaging, or single-cell methods. With this tool we are able to address many unanswered questions in cell biology, such as how direct protein degradation compares to gene knockout in uncovering protein functionality, or how stress responses compare when unfolding different proteins in different subcellular environments. Ultimately, this tool represents a platform to enable scalable, multimodal proteomic studies.

The Role of KDM6A/B Histone Methyltransferases in Pancreatic Islet Maturation and Function

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H3K27me3 is associated with transcriptional repression, while H3K4me3 is associated with transcriptional activation. KDM6A and KDM6B specifically demethylate H3K27. β cell functional genes are monovalently marked with H3K4me3 in β cells and bivalently marked with H3K4me3 and H3K27me3 in α cells. Furthermore, KDM6B loss during early pancreatic development results in decreased β cell mass and impaired glucose tolerance. We hypothesized that H3K27 demethylation in endocrine progenitor cells and the β cell lineage is important for proper β cell differentiation, maturation, and insulin secretion.

KDM6A/B were deleted in endocrine progenitor cells (islet KO) or β cells (β cell KO). Islet KO, β cell KO and control littermate mice were evaluated for plasma glucose and insulin, islet insulin secretion, endocrine cell proportions, and islet size at 2 and 12 months of age. Male islet KO mice exhibited impaired glucose tolerance and decreased plasma insulin concentrations at 2 months, which progressed to diabetes by 12 months. β cell KO mice had no differences in glucose tolerance or plasma insulin at 2 or

12 months. However, continuous peri-fusion assays on whole islets from male β cell KO mice demonstrated reduced glucose-stimulated insulin secretion at 2 and 12 months. Islet KO and β cell KO mice had no difference in endocrine cell mass or proportions or islet size at 2 or 12 months. KDM6A/B deletion in endocrine progenitor cells led to glucose intolerance and diabetes, while loss of KDM6A/B in β cells led to impaired glucose-stimulated insulin secretion. Therefore, H3K27 demethylation is not required for β cell differentiation but is necessary for proper β cell function, and the timing of loss of KDM6A/B activity during cell β development correlates with phenotype severity.

Parsing out Polymicrobial Interactions during Clostridioides difficile Infection

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The majority of hospital-acquired infections in the United States are caused by Clostridioides difficile. C. difficile infection (CDI) causes a spectrum of disease from diarrhea to severe gastrointestinal (GI) complications and/or death. Our goal is to understand how the resident gut microbial community influences outcomes of CDI. To that end, we isolated 71 gut bacteria from 15 CDI patients. The most common isolates were *Enterobacteriaceae*, a family which normally occupies a small niche in the healthy anaerobic gut. During CDI, a robust inflammatory response, damage to the host epithelium, and the unique metabolism of *C. difficile* results in an altered metabolic environment. *Enterobacteriaceae* flourish in inflammatory states, but little is known about their fitness in the CDI gut. Additionally, the specific impact of diverse members of this family during CDI is not well understood. We hypothesize that *Enterobacteriaceae* 1) can influence *C. difficile* behavior and 2) have increased fitness in the gut during CDI. To test this, we evaluated *C. difficile* growth and toxin production in cell-free conditioned media from *Enterobacteriaceae* isolates grown anaerobically or anaerobically. We observe that *C. difficile* toxin production increases in conditioned media from anaerobic, but not aerobic conditions. In a parallel approach, we are testing how conditioned media from *C. difficile* impacts *Enterobacteriaceae* virulence factors. Finally, we will use transposon mutant libraries of key species in mouse models of CDI to determine the genetic requirements for expansion in the GI tract. These approaches will identify the metabolic cues mediating interspecies interactions and provide mechanistic understanding of how *Enterobacteriaceae* influence CDI disease.

Paradoxical Reaction to Benzodiazepines (PROBE) in Children: Computational Phenotypes

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Many children undergoing medical procedures require sedation for anxiolysis, amnesia, and patient safety. Benzodiazepines (BZDs) are commonly prescribed. Despite their efficacy and favorable risk profiles, BZDs may trigger paradoxical reactions. The underlying mechanisms of these reactions are unknown. Here, our aims were: (1) create electronic medical record (EMR)-based computational phenotypes of BZD paradoxical reactions; (2) determine the enterprise-wide prevalence of the BZD paradoxical reaction phenotype at the Children's Hospital of Philadelphia (CHOP); and (3) identify genetic variants that are associated with the BZD paradoxical reaction phenotype. We used the CHOP's Arcus Database. Via EMR, we will identify children who received BZDs and documentation of a paradoxical reaction (cases) or no paradoxical reaction (controls) to create computational phenotypes. BZD allergy flags and ontological terms derived from CHOP clinical content experts, have been compiled for natural language processing of clinical notes. Identified cases and controls will be linked to patient-specific genotyped data previously collected from the Center for Applied Genomics. Analysis will include linear regression models to determine individual factors associated with BZD paradoxical reactions. To date, we have identified 891 children with the BZD paradoxical phenotype by allergy flag alone. Children who present with a paradoxical reaction allergy flag and a corresponding benzodiazepine administration will be considered cases. Clinical notes are being assessed. We aim to identify novel genetic loci associated with the BZD paradoxical reaction phenotype. Identification of these novel loci prior to BZD administration may avoid adverse events

A novel twist on a canonical neuromuscular junction development pathway

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Development of the neuromuscular junction (NMJ) is coordinated by the motor axon and the muscle fiber. Canonically, axonally-released Agrin binds its receptor Lrp4 on the postsynaptic muscle membrane to activate MuSK and induce clustering of acetycholine receptors (AChR). Mutants in Agrin, lrp4, and MuSK fail to form NMJs in mice and within the larval zebrafish trunk. Thus, we were surprised to observe that lrp4 and Agrin mutants have a distinct NMJ phenotype within the zebrafish pectoral (pec) fin. While pec fins in siblings have ~300 small, evenly dispersed AChR clusters, lrp4 or Agrin mutant pec fins have ~75 very large AchR clusters and abnormal swellings in the motor axon innervation pattern. This enlarged NMJ phenotype in mutant pec fins is in striking contrast to the miniature NMJs within the trunk muscles of the exact same mutant larvae. Ablation of motor neurons prior to axon outgrowth into the pec fin in wildtype animals to eliminate all axon-derived signals also phenocopies lrp4 and Agrin mutants, suggesting that pec fin muscles are predisposed to form large AChR clusters that are dispersed via Lrp4/Agrin signaling upon arrival of the axon. As Agrin and Lrp4 canonically activate MuSK, we were again surprised that MuSK mutants do not share the lrp4/Agrin mutant pec fin phenotype. Instead, reducing MuSK suppresses the lrp4 mutant pec fin phenotype, suggesting that MuSK is paradoxically overactive in lrp4/Agrin mutants. We are now testing if Wnts, which can activate MuSK and can be inhibited by Lrp4/Agrin in other contexts, may drive the over-clustering of AchRs and axon swelling defect within mutant pec fins. Thus, we have uncovered a dogma-challenging pathway in which Lrp4 and Agrin restrain MuSK signaling to distribute AChRs during NMJ development.

A novel role for X-Chromosome Inactivation in regulating T cell responses to Influenza

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Females mount stronger immune responses for various infectious diseases caused by viruses and bacteria. One important example is influenza infection, where pre-puberty and elderly males are most susceptible, yet pre-menopausal women are more at risk compared to men of similar age. Sexual dimorphism in immune responses has both hormonal and genetic origins, and while sex hormone signaling has been implicated in differential susceptibility to influenza, the genetic contribution is poorly understood. The X Chromosome is enriched for immunity-related genes, and female mammals have two X chromosomes and use random X-Chromosome Inactivation (XCI) to normalize X-linked gene expression between the sexes. XCI is established early in female embryonic development and maintained through each cell division by the lncRNA Xist, which coats the inactive X (Xi), and various repressive histone modifications. In contrast to most somatic cells, resting T cells display altered Xist RNA localization, with lack of Xist RNA clouds or repressive histone modifications on the Xi, which dynamically relocalize to the Xi upon activation. We hypothesize that perturbations to XCI maintenance in T cells will affect T cell function and responses to influenza infection in mice. Our preliminary results indicate that deletion of Xist in T cells results in female-specific increased susceptibility to influenza infection. Our results will reveal the role for XCI gene escape in T effector populations important for protection against influenza, which likely contribute towards sex differences with immune responses.

Reinnervated motor units and slow type of skeletal muscle decide selective resistance to ALS-related motor unit degeneration in a mouse model of TDP-43 proteinopathy

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Surviving motor neurons (MNs) can compensate for the loss of vulnerable neural circuits in amyotrophic lateral sclerosis (ALS) patients. However, the precise nature of the selective vulnerability of motor units and the reinnervated motor units, to compensate for the loss of motor units during the Tar-DNA binding protein-43 (TDP-43)-triggered ALS disease course, remain unclear. This study demonstrates that the inducible human TDP-43 pathology transgenic mice used in the present study can recover motor function via motor units that are reinnervated by approximately 40% of initial motor unit pools and 60% of newly adjacent ones. Notably, reinnervated motor units from the adjacent MN pool in fast-type skeletal muscle have resistance to axonal dieback during a second human Tar-DNA binding protein-43 (hTDP-43)-triggered disease while most initial fast-type motor units have a vulnerability in response to the first hTDP-43-triggered disease. Moreover, cross-reinnervation surgery, which generates two different newly cross-connected subnerves, was employed to investigate whether a specific type of MNs and/or reinnervated MNs are intrinsically disease-resistant. Finally, reinnervated MNs have selective rather than intrinsic resistance of a specific type of MNs. Additionally, a specific muscle fiber type, but not a specific MN type, can determine the selective vulnerability or resistance during mutant hTDP-43-triggered disease via the morphological difference of neuromuscular innervation.

Pre-innervated tissue-engineered muscle promotes a pro-regenerative microenvironment following volumetric muscle loss

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Volumetric muscle loss (VML) is the traumatic or surgical loss of skeletal muscle beyond the inherent regenerative capacity of the body, generally leading to severe functional deficit. Formation of appropriate motor innervations remains one of the biggest challenges for both autologous grafts as well as tissue-engineered muscle constructs. In the present study, we address this challenge by developing pre-innervated tissue-engineered muscle comprised of long aligned networks of spinal motor neurons and skeletal myocytes on aligned nanofibrous scaffolds. Motor neurons led to enhanced differentiation and maturation of skeletal myocytes in vitro on aligned nanofiber sheets. These pre-innervated tissue-engineered muscle constructs when implanted in a rat VML model significantly increased satellite cell density, neuromuscular junction maintenance, graft revascularization, and muscle volume over three weeks as compared to myocyte-only constructs and nanofiber scaffolds alone. These pro-regenerative effects may enhance functional neuromuscular regeneration following VML, thereby improving the levels of functional recovery following these devastating injuries. Ongoing studies are directed towards fabricating human iPSC derived neuromuscular constructs and evaluating chronic functional efficacy in a clinically relevant model of VML.

Suppression of Experimental Myasthenia Gravis by Antibodies to the Cytoplasmic Domain of Muscle Acetylcholine Receptor

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Myasthenia gravis (MG) and experimental autoimmune myasthenia gravis (EAMG) are caused by antibody-mediated autoimmune responses to muscle nicotinic acetylcholine receptors (AChRs) that impair neuromuscular transmission, causing muscle weakness. We have developed a therapeutic vaccine for EAMG in rats comprising bacterially-expressed cytoplasmic domains of human AChR subunits that prevent the development of chronic EAMG and rapidly suppress ongoing EAMG. We hypothesize that the therapy acts at least partially by feedback suppression mediated by antibodies to the vaccine. We found that passive transfer of total IgGs purified from rats immunized with the therapeutic vaccine permanently reduced pathological autoantibodies to the AChR extracellular epitopes, while administration of IgG from normal rats only transiently inhibited antibody production. The treatment incrementally reduced development of chronic EAMG, but the reduction was not statistically significant compared to the normal IgG control. The therapeutic effects of antibodies elicited by the therapeutic vaccine will be further evaluated in the current study by passively transferring antigen affinity-purified antibodies, rather than total IgG, into rats with EAMG. This will extend our observations beyond those of intravenous immunoglobulin (IVIg), predicated on the administration of total IgG, and allow us to explore feedback suppression mediated by antigen-specific antibodies.

A Bitter Death: Nuclear-Localized Bitter Taste Receptors Activate Calcium Signaling and Apoptosis

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Taste family type 2 receptors (T2Rs) are G-protein coupled receptors (GPCRs) involved in bitter taste. T2Rs are also expressed outside the tongue, where they function in diverse roles. In upper-airway epithelia, T2Rs are localized to the plasma membrane and function to detect bitter bacterial quorum-sensing molecules, thereby activating innate immune responses. However, through both immunofluorescence and immunoblotting, we have found that many endogenously expressed T2Rs of the lower-airway epithelia are localized to the nucleus. Nuclear GPCRs are known to be important in some cell types, but nuclear GPCR signaling is not well studied. Many bitter compounds, including bacterial quorum-sensing molecules, are hydrophobic and likely cell permeant; thus, some bitterants may function to activate nuclear-localized T2Rs. Using genetically encoded nuclear-targeted fluorescent indicators, we found that some T2R agonists elevate nuclear calcium and are sensitive to the phospholipase C (PLC) inhibitor U73122, consistent with canonical taste signaling (Gα gustducin and Gβγ activation of PLC). Here, we demonstrate that bitterants induce nuclear calcium release and activate apoptosis in lower airway epithelial cells.

A genome-first approach to liver and cardiometabolic phenotypes and survival in MARC1 p.Ala165Thr (rs2642438) carriers

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A common coding variant in the mitochondrial amidoxime reducing component 1, MARC1 (rs2642438; p.Ala165Thr), was recently found to be associated with protection from cirrhosis in individuals of European ancestry. However, its impact on liver and cardiometabolic phenotypes as well as overall and cause-specific mortality remains elusive. Using a genome-first approach, we explored a range of phenotypes associated with MARC1 p.Ala165Thr in the UK Biobank and the Penn Medicine BioBank a (PMBB). MARC1 p.Ala165Thr was significantly associated with higher triglycerides, lower total cholesterol, lower LDL-C, lower apolipoprotein B, lower HDL-C, lower apolipoprotein A-I, higher lean mass, and higher IGF-1. Per each minor allele, the risk of nonalcoholic fatty liver disease (NAFLD) was reduced by ~15%. In African-American individuals in the PMBB, the allele frequency of MARC1 p.Ala165Thr was lower, but carriers showed protection from NAFLD and the same distinctive lipid phenotype. Importantly, MARC1 p.Ala165Thr carriers did not show higher cardiovascular disease burden. In prospective analyses, the homozygous minor allele was associated with up to 39% lower rates of liver-related mortality in the general population, while no risk of increased cardiovascular death could be observed. Strikingly, liver-related mortality was more than 50% reduced in diabetic participants or carriers of the patatin-like phospholipase domain-containing-3 (PNPLA3) gene rs738409:G. Together these data highlight MARC1 as an important genetic liver disease modifier that also influences plasma lipids in a gene dose-dependent manner. Our data point toward potential physiological mechanisms and reveal a remarkable association with liver-related mortality calling for future studies that explore its therapeutic potential.

The influence of age of first concussion on visio-vestibular function in adolescent patients

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History of head impact exposure in adults is linked to greater risk of subsequent concussion and long-term impairments. Research has focused on adults, with little data examining younger patients. Therefore, the purpose of this study is to investigate how age of first concussion (AFC) influences visio-vestibular examination (VVE) acutely following a subsequent concussion in adolescents. Data were collected from patients aged 14-18 years with a concussion history presenting at their first injury visit (≤28 days from injury) for a subsequent concussion. Demographics were self-reported prior to the start of the exam. The VVE consisted of 9 subtests: smooth pursuit, horizontal/vertical saccades and gaze stability, binocular convergence, monocular accommodation, and complex tandem gait. Primary outcomes included AFC, lifetime concussions (2 vs. 3+), VVE subtests (normal/abnormal), and total VVE score (abnormal = 2+ abnormal subtests). We used multivariable logistic regressions to determine if AFC was associated with abnormal VVE outcomes while controlling for sex, age, and lifetime concussions. One hundred and ninety-one patients (male = 67 (35.1%); age = 15.7 3 1.3 years, lifetime concussions = 2.4 3 0.7) self-reported an average AFC of 13.2 3 2.1 years. Younger AFC was significantly associated with greater odds of abnormal vertical saccades (OR = 0.82; 95% CI = 0.69, 0.98) and trended toward significance for horizontal saccades (OR = 0.85; 95% CI=0.72, 1.00). No other associations with AFC were found. Post-concussion visio-vestibular function appears to be unaffected by AFC, with the exception of saccadic eye movement. This study provides formative data regarding the age of prior concussion on clinical observations acutely post-concussion.

What is the role of myeloid-derived suppressor cells in surgery-induced suppression of photodynamic therapy efficacy?

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Lung-sparing surgical tumor debulking with intraoperative photodynamic therapy (PDT) promisingly extends survival for patients with malignant pleural mesothelioma (MPM). Unfortunately, most people treated with this approach develop local tumor recurrence, so it is crucial to determine the potential mechanisms that prompt treatment failure and identify mitigation strategies. Surgery alone is known to induce inflammation. We observed in our pre-clinical models of murine MPM treated with simulated surgery (tumor injury without cytoreduction) followed by PDT that surgery diminishes the curative potential of PDT. To further explore the mechanisms by which surgically induced inflammation might diminish PDT efficacy, we used these murine MPM tumor injury/PDT models to determine key leukocyte players in the pathway from local tumor response to establishment of long-term systemic control. Using flow cytometry-based immunophenotyping and functional studies focusing on myeloid-derived suppressor cells and T cells, we have found markedly different patterns of innate and adaptive inflammatory cells in tumors, tumor-draining lymph nodes, and spleens of MPM tumor bearing animals. Overall, these studies suggest that surgically mediated modulation of immune cell trafficking and functionality prior to PDT lead to a systemic suppression of PDT-induced anti-tumor immunity. Targeted inhibition of these molecular or cellular signals of surgically induced inflammation can potentially restore PDT efficacy in the intraoperative setting.

Hypoxia-induced Collagen VI modification weakens endothelium and promotes metastasis

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Metastasis is the leading cause of cancer-related deaths. However, the mechanisms behind the metastatic cascade remains poorly understood. Tumor's metastatic potential is strongly influenced by microenvironmental cues such as low oxygen (hypoxia). Our published work reported that lung metastases in sarcoma are associated with increased primary tumor expression of the hypoxia-inducible collagen-modifying lysyl hydroxylase, PLOD2. In multiple subtypes of sarcoma, excessive collagen lysyl hydroxylation results in secretion of immature collagen aggregates able to physically associate with tumor cells and promote both intravasation and extravasation. In the current study, we identify collagen VI as a putative substrate of PLOD2, and we demonstrate that PLOD2-modified collagen VI weakens the endothelial barrier and promotes transendothelial migration. In mice, collagen VI is critical for distant metastasis. Clinically, the expression of both PLOD2 and collagen VI is correlated with poor disease outcomes. Our study identifies a novel mechanism of sarcoma lung metastasis, opening up opportunities for therapeutic intervention.

Direct binding of ESRP1 to regulated transcripts is required for position-dependent splicing regulation

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Coordinated regulation of alternative splicing is essential to the establishment of cell identity. The Epithelial Splicing Regulatory Proteins (Esrps), ESRP1 and ESRP2, are highly conserved paralogous proteins required for organogenesis of multiple organ systems. Compromised function of Esrps contributes to human diseases and pathologies. Esrps are robustly expressed in the epithelial cells of the epidermis, large and small intestines, salivary glands, the stomach, and a variety of other tissues, where they are vital in promoting an epithelial splicing network. Although ESRP1 and ESRP2 share partial functional redundancy, ESRP1 appears to play a larger role in regulating gene expression.

We generated a map of ESRP1 binding to RNA using a combination of enhanced immunoprecipitation coupled with high throughput sequencing (eCLIP) in the epithelial cells of mouse epidermis, as well as RNA sequencing analysis of alterations in splicing and total gene expression after epidermal ablation of ESRP1 and ESRP2. We show that ESRP1 regulates splicing primarily through direct binding in a position-dependent manner to promote either exon inclusion or skipping. In particular, we show that ESRP1 binding upstream of or within alternatively spliced exons suppresses exon inclusion. However, binding downstream of the non-constitutive exon promotes exon inclusion. In addition, we identified widespread binding of ESRP1 in 3' and 5' untranslated regions (UTRs) of genes enriched for epithelial cell function.

Role of Akt signaling in the regulation of glucose homeostasis and skeletal muscle physiology

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Skeletal muscle (SkM) accounts for a majority of glucose disposal following a meal. Defects in the insulin signaling pathway in SkM have been hypothesized to be a primary cause of insulin resistance. The serine/threonine kinase AKT is a central regulator of insulin action and a decrease in AKT activity is observed in muscles from insulin-resistant mice and humans.

To test the direct requirement of SkM AKT signaling on systemic glucose metabolism and muscle physiology, we generated several mouse models of SkM AKT deficiency.

Unexpectedly, mice lacking AKT2 alone exhibited normal insulin signaling, insulin sensitivity, and muscle mass despite a dramatic reduction in phosphorylated muscle AKT. In contrast, deletion of both muscle AKT isoforms (M-AKTDKO) resulted in a complete loss of AKT-mediated insulin signaling. Despite the lack of AKT activity, M-AKTDKO mice were insulin sensitive and displayed normal rates of glucose uptake in response to insulin. This chronic loss of AKT was associated with mitochondrial dysfunction and subsequent AMPK activation, which we identified to be an important regulator of muscle insulin sensitivity. Unlike glucose metabolism, loss of muscle AKT resulted in muscle atrophy and defective muscle performance. Mechanistically, activation of mTORC1 and inhibition FOXO1 were both required and sufficient to induce muscle hypertrophy in the absence of AKT.

Collectively, these data define the cellular pathways responsible for AKT's control of muscle growth and function. Moreover, we made the surprising discovery that AKT is not an obligate intermediate for insulin-stimulated glucose uptake in all conditions, which suggests the existence of additional insulin-dependent AKT-independent signaling pathways for the regulation of glucose homeostasis.

Microtubules orchestrate local translation to enable cardiac growth

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Post-mitotic muscle cells of the heart undergo constant contraction. These cells must maintain homeostasis of the contractile unit called sarcomere while also retaining the ability to remodel. This is important during growth, when the cardiomyocyte (CM) requires the addition of new sarcomeres. Yet, it is still unclear how new sarcomeres are formed, added, and remodeled within the existing structure. Although the transcriptional landscape of cardiac hypertrophy has been studied extensively, there is little information regarding spatiotemporal regulation. We aim to uncover the mechanisms of mRNA transport and their role in hypertrophy. Our data suggest that microtubules are required for proper trafficking of mRNA in the CM. We used smFISH to find that transcripts are enriched at the sarcomere and intercalated disc of the CM as reported previously. Treatment with the microtubule-destabilizer colchicine, both *in vitro* and *in vivo*, causes a perinuclear collapse of mRNA. Proper localization is also disrupted by kinesin-1 knockdown. We find that a growth stimulus (phenylephrine, PE) induces a peripheral localization of sarcomeric transcripts in growing cells, but both this specific localization and growth of the cell are blocked in the presence of colchicine. Additionally, we demonstrate that ribosomes and nascent proteins also localize to the sarcomere both *in vitro* and *in vivo*, and this localization is similarly disrupted with colchicine treatment. Concomitant with mislocalized translation, protein degradation is increased in the presence of colchicine. We hypothesize that the CM uses a strategy of local translation whereby transcripts are actively transported to distal locations in the cell and locally translated by ribosomes for insertion into new sarcomeres.

Genomic characterization of RNA granule-linked genes using large biobanks

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Diseases that impact the nervous, musculoskeletal, or immune system represent important disease categories with overlapping etiologies. We suggest studying genetic similarities in the context of all three disease categories may provide novel insights. Evidence has recently been growing that each of these disease categories can have underlying pathologies that involve RNA granule formation. These granules are transient aggregates in the cytoplasm made up of weakly interacting RNA and protein molecules for regulating translation and mRNA turnover. We quantified the potential capacity for RNA granule-linked genes to be associated with disease by leveraging data from large biobanks. We used a list of 4,520 RNA granule-linked genes from a published systematic review, 358 of which were considered "gold-standards" due to the overwhelming evidence. Using the Genome Aggregation Database (gnomAD), we found that RNA granule-linked genes were significantly less likely to tolerate loss-of-function variants ($p < 2.2e-16$), likely due to an enrichment for essential genes ($p = 1.5e-268$). The gold-standard genes were enriched for disease ontology terms such as "Neuro-degenerative disease", "Amyotrophic Sclerosis" and "Influenza" (FDR < 0.05). We performed a targeted Phenome-wide association study using 32,268 individuals of European ancestry from the UK Biobank with whole-exome sequencing data and disease codes related to central nervous system, musculoskeletal, and immune system disorders. We found the burden of rare missense variants (MAF < 0.01) in the gene OGG1 reached Bonferroni significance with the disease code for "Osteoarthritis of knee" ($p = 2.62e-8$). Thus, studying genetic variants in RNA granule-linked genes provides a new context for studying these three disease categories.

The Mitochondrial Calcium Uniporter Affects Pancreatic Cancer Development and Invasion

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Cancer is one of the top two causes of death in the United States. While many cancers respond well to newer targeted therapies, pancreatic ductal adenocarcinoma (PDAC) still has a very poor prognosis which is partially attributable to early metastasis contributing to mortality. We previously showed that cancer cell lines may be "addicted" to constitutive uptake of Ca^{2+} by mitochondria through the mitochondrial calcium uniporter (MCU) at endoplasmic reticulum-mitochondria contact sites. We hypothesized that mitochondrial Ca^{2+} influx through MCU contributes to cancer development, proliferation, and metastasis in PDAC. We here report that high MCU expression is associated with KRAS mutations, which commonly drive PDAC, as well as poor survival outcomes. We employed the Pdx1cre; KrasLSL-G12D/+; $p53^{fl/+}$; Rosa26LSL-YFP/LSL-YFP; $Mcu^{fl/fl}$ (KPCY) murine model of PDAC, generating KPCY-McuKO animals and cell lines from their YFP-positive tissues for further analysis. Cell lines developed from KPCY-McuKO pancreatic tissues lacked mitochondrial Ca^{2+} uptake, which was rescued by stable re-expression of MCU. MCU re-expression increased cell motility, self-renewal, and proliferation. It also associated with fibroblastic morphology, decreased surface expression of e-cadherin and increased morphological response to TGFβ, indicative of partial epithelial-to-mesenchymal transition (EMT). In an immunocompetent orthotopic model of PDAC, MCU knockout ablated tumor growth and metastasis in a manner reversed by stable re-expression of MCU. Our findings suggest MCU may contribute to growth and metastasis, potentially through partial EMT. In conclusion, MCU-mediated mitochondrial Ca^{2+} uptake contributes to PDAC development and metastasis and may present a therapeutic target for cancer treatment.

CAR-T Cells Targeting Cancer-Specific Splice Isoforms of Fibronectin Exhibit Potent Anti-Tumor Activity

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Current advances in cellular immunotherapy, specifically CD19 CAR-T cell therapy, have revolutionized the treatment of B cell malignancies; however, the success of this approach in solid tumors remains the greatest unmet burden. CAR-T cells have lacked solid tumor efficacy due to a number of challenges, including scarcity of tumor-specific antigens, poor expansion and persistence, and physical barriers to T cell infiltration caused by the dense, immunosuppressive tumor microenvironment (TME). Targeting of post-translational modifications that occur exclusively in and around transformed cells can be a means to target immune activation specifically within tumors. Previous work in our lab demonstrated remarkable tumor regression across many cancer histotypes after treatment with TnMUC1 CAR-T cells, which target an abnormal glycoform present on the surface of many cancers. The present work demonstrates both in vitro and in vivo efficacy of CAR-T cells targeting cancer-specific isoforms of the extracellular matrix (ECM) protein fibronectin (FN). CAR-T cells targeting oncofetal FN, an abnormal glycoform of FN, are activated by androgen insensitive PC3 and DU145 prostate cancer cell lines, secrete high concentrations of IFN-γ, and are lytic of tumor cells. Additionally, FN-targeting CAR-T cells promote rapid tumor rejection in a subcutaneous PC3 xenograft model. Immunohistochemical analysis of PC3 tumors harvested from mice revealed significant T cell infiltration of FN-CAR-T and TnMUC-1 CAR-T cells compared to tumors from mice treated with NTD T cells, which were primarily excluded from tumor beds. This data provides a novel cancer immunotherapy approach, targeting an ECM protein, for the treatment of prostate tumors and potentially other cancer histotypes.

Assessment of race-based linguistic differences in physician notes of high use patients using automated text mining

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Clinicians, similar to the general population, possess implicit biases against individuals from racial or ethnic minority groups. Despite this pervasive reality, the direct effects of biases on health care outcomes are poorly understood. Prior research investigating the effects of clinician bias have been limited by small sample sizes, low clinician response rates, and the use of vignettes rather than real-world data. It is important to investigate implicit bias in real-world settings because bias is more likely to occur, and affect behavior, in settings of high cognitive load, limited time, and clinical ambiguity. To fill this gap in the literature, we will study the variability in clinician sentiment among high use patients by race. High use patients were selected as the population for study because they often face stigmatizing illnesses and their social and medical complexities present clinical uncertainty for clinicians, which makes them more susceptible to bias. We will perform a single-center retrospective cohort study of all inpatient encounter notes for high use patients written by clinicians while admitted to the University of Pennsylvania Health System during calendar year 2019. Using both open- and closed-vocabulary text-processing methods, we will identify vocabulary terms in the text of encounter notes, classify terms using a sentiment lexicon, assess linguistic differences associated with patients' race, and measure their association with clinical outcomes. The results of this study will contribute to our understanding of implicit bias in real-world settings, provide information about the mechanism between implicit bias and clinical outcomes, and offer a basis for future research needed to advance interventions for implicit bias.

Maternal Macronutrient Consumption Quantified in Late-Gestation Pregnant Mice: Implications for Fetal Metabolism

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Maternal macronutrient intake increases to meet demand for fetal biomass accretion and maternal health, but an integrated understanding of whole-body macronutrient metabolism during pregnancy is lacking. Stable isotope tracer studies can yield valuable quantitative information about substrate utilization in vivo. We have measured macronutrient turnover kinetics for five nutrients simultaneously in late-gestation pregnant mice and non-pregnant controls by infusing ^{13}C- or ^{2}H-labeled glucose, lactate, glutamine, valine, and β-hydroxybutyrate. Glucose and lactate were found to have the highest circulatory turnover fluxes of the macronutrients tested, with a greater increase in pregnant mice. The fluxes of amino acids and β-hydroxybutyrate were also higher in pregnant mice, consistent with the accelerated fasting response in late gestation which increases circulating amino acids and ketone bodies during acute nutrient deprivation. Recent studies in late-gestation pregnant mice have revealed that maternal metabolite milieu greatly affects fetal metabolism to match maternal nutritional status. Interestingly, we have identified metabolic pathways for amino acid synthesis active in the fetus that are not contributed by maternal circulating metabolites but are occurring in a fetal autonomous manner. This work provides a quantitative framework for understanding systemic metabolism during pregnancy and identifying signals that contribute to the regulation of maternal nutrient partitioning. A better understanding of maternal-fetal metabolic communication can inform diagnostic and therapeutic options for metabolic diseases of pregnancy including gestational diabetes and intrauterine growth restriction.

Treatment of CNS lysosomal storage disorders by fusion of TTC and lysosomal enzymes

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Lysosomal storage diseases form a group of multi-system metabolic disorders that result from defects in genes coding for proteins pertinent to lysosome function and maintenance. Current treatments have shown efficacy in many organ systems, however correction or attenuation of disease in the central nervous system (CNS) has been more difficult to achieve. This work aims to improve AAV gene therapy strategies for delivery of a therapeutic gene to the CNS by increasing the distribution of the protein in the brain. To accomplish this we utilize TTC, the c-terminal fragment of the tetanus toxin protein (TeNT), to improve distribution of therapeutic enzyme by transference of TeNT's retrograde axonal and trans-synaptic mobility onto a conjugated enzyme. We chose to investigate the use of TTC in a mouse model of MPS VII, which is caused an attenuation of β-glucuronidase (GUSB) activity. We determined that for many lysosomal enzymes, fusion with TTC would generate a sequence too large to fit into the AAV packaging limit. Thus, we generated a series of truncated TTC fragments and tested them in vitro for activity. The most promising variant (HCC) was used to generate an AAV vector along with the full length TTC. Mice were administered vector into the hippocampus. Distribution of GUSB.TTC, GUSB.HCC, and GUSB was analyzed by activity stain on brain sections. Results indicated that truncations reduced activity of GUSB by ~50-70% GUSB. The fusion of TTC greatly improved distribution of GUSB throughout the brain. While the HCC truncation improved distribution, it was not to the extent of full length TTC. Our results indicate TTC and smaller variants could improve gene therapy for lysosomal storage disease by increasing distribution and allowing for a smaller minimal effective dose.

ROS induced activation of NLRP3 inflammasome

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Inflammation is a well-organized protective response to pathogens and consists of immune cell recruitment into areas of infection. Inflammation either clears pathogens and gets resolved leading to tissue healing or remains predominantly unresolved triggering pathological processes in organs. Among the major players of the inflammation cascade is the NOD like receptor protein 3 (NLRP3) inflammasome which is activated in both infection and non-infection based models of inflammation. However, the exact mechanisms by which the NLRP3 inflammasome is activated is not clear. We hypothesize ROS generated with inflammation is a trigger for the NLRP3 inflammasome expression and activation. Infection based in vitro models of inflammation were generated by treating various cells types (pulmonary endothelium and human gingival fibroblasts [HGF] from human sources) with lipopolysaccharide (LPS). HGFs taken from two different patients were treated with LPS from *P. gingivalis* for 24 hrs. Both ROS and NLRP3 were assayed by fluorescence microscopy and immunostaining, respectively. ROS production was detected by staining the cells with the fluorescent dye CellRox, whereas the activation of NLRP3 inflammasome and caspase-1, was detected by staining with primary antibody for NLRP3 and caspase-1 respectively. Non infection based model used was that of ischemia/reperfusion to mimic lung transplant. ROS production post stimuli associated significantly with NLRP3 as detected by monitoring expression and activity of caspase-1. ROS induces the activation of the NLRP3 inflammasome, but further research is needed to show the link between the intermediate signals (kinases and phosphatases) that drive expression and activation of the inflammasome.

Control of FoxP3+ Treg Production, Stability and Function by the Nuclear Co-regulator, Sin3a

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While Foxp3$^+$ T-regulatory (Treg) cells are well established as a crucial component of immune homeostasis and self-tolerance, contributions of large, multiprotein complexes that regulate and program their gene expression remain obscure. To address this, we evaluated the transcriptional repressor, Sin3A, by evaluating its conditional deletion in Foxp3$^+$ Tregs. Sin3A$^{-/-}$FoxP3^{Cre} mice developed severe, systemic autoimmunity with greatly enlarged superficial lymph nodes and massive inflammatory lesions in lungs, skin, livers and kidneys. These mice developed a range of autoantibodies to islet cells, striated muscles, keratin, endomysium and gastric parietal cells. Proportions of CD4$^+$ FoxP3$^+$ Tregs were significantly decreased in all peripheral lymphoid organs, especially in superficial lymph nodes. Ratios of T effectors to Tregs, and follicular T effectors to follicular Tregs were drastically increased within spleens and superficial lymph nodes. Sin3A deficient Tregs produced significant amounts of pro-inflammatory cytokines such as Granzyme B, IFN-γ and IL-2. We also found that Sin3A was essential for peripheral iTreg conversion since conventional CD4$^+$CD25$^-$ cells from KO mice completely lacked the ability to convert to FoxP3$^+$ iTregs ex vivo in the presence of IL-2 and TGFβ. Lastly, Tregs treated with a pharmacologic Sin3A inhibitor, Selamectin, displayed a dose-dependent reduction of suppressive activity, while Selemectin-treated wild-type CD4$^+$CD25$^-$ cells had significantly reduced iTreg conversion. These data show that Sin3A functions in regulating Treg gene expression and maintains the unique properties of these key immune cells. In addition, these data indicate a potential for therapeutic modulation of Tregs by pharmacologic targeting of components within the Sin3A complex.

Structure of the ancestral TRPY1 channel from *Saccharomyces cerevisiae* reveals mechanisms of lipid and calcium modulation

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Transient Receptor Potential (TRP) channels have evolved in mammals and other higher organisms to control various cellular functions in response to a wide variety of chemical and physical stimuli. This large and diverse family of eukaryotic channels first emerged in fungi where they are mainly responsible for osmoregulation and are considered to be mechanosensitive. The *Saccharomyces cerevisiae* vacuolar transient receptor potential yeast 1 (TRPY1) is the most studied TRP channel from fungi, but the molecular details of channel modulation remains elusive so far. Here, we describe the full-length cryo-electron microscopy (cryo-EM) structure of TRPY1 at 3.1 Å resolution. The structure reveals a unique architecture for TRPY1 among all eukaryotic TRP channels with an evolutionarily conserved and archetypical transmembrane domain, but distinct structural folds formed by cytoplasmic N- and C-termini. We identified the inhibitory phosphatidylinositol 3-phosphate (PI(3)P) lipid binding site, which shed light into the lipid modulation of TRPY1 in vacuolar membrane. We also elucidated two Ca^{2+} binding sites: one in the cytoplasmic side, implicated in activation and the other in the vacuolar lumen side, involved in channel inhibition. These findings together with data from molecular dynamics simulations provide structural insights into understanding the basis of TRPY1 channel modulation by Ca^{2+} and lipids.

Engineered antigen-expressing CAAR-T cells to target autoreactive B cells

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Acquired thrombotic thrombocytopenic purpura (aTTP) is a life-threatening thrombotic microangiopathy characterized by abnormal bleeding and clotting due to dysregulated platelet activation. aTTP results from an autoantibody response directed against a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS13), a plasma protease that cleaves von Willebrand Factor (vWF). Under normal physiological conditions, and in the absence of endothelial injury, the length of vWF multimers is regulated through cleavage by ADAMTS13 and this process prevents pathological platelet activation and clotting. The pathological activation of autoantibodies directed against ADAMTS13 results in severely reduced activity of ADAMTS13 leading to the clinical and pathological features of TTP. To develop a curative treatment, we developed chimeric auto-antigen receptors (CAARs) to target ADAMTS13-specific B cells responsible for the production of the autoantibodies that cause aTTP. We generated receptors employing different combinations of ADAMTS13 domains as extracellular CAAR domains linked to intracellular signaling domains derived from T cell or NK cell receptors. ADAMTS13-based CAAR-T cells specifically killed target cells expressing human autoantibodies directed at the cysteine-rich spacer domain (CS) of ADAMTS13, a region known to contain enzyme-inhibitory epitopes. The potency of the CAAR-T cells was comparable to that of a CD19-directed CAR-T. In the presence of anti-CS expressing target cells, we observed CD107 degranulation and cytokine production. ADAMTS13 CAAR-T cells also proliferated in the presence of anti-CS expressing targets. These results suggest that ADAMTS13-based CAAR-T cells may be an approach to curing aTTP and support further testing in *in vivo* models.